

# *Acta Medica Okayama*

---

*Volume 13, Issue 4*

1959

*Article 5*

DECEMBER 1959

---

## Cryptococcus, Pathological observations of five autopsy cases and one biopsy case

Katsuo Ogawa\*

Akira Uejima<sup>†</sup>

Tsuyoshi Inohara<sup>‡</sup>

Kiyoshi Kuroda\*\*

Joji Murase<sup>††</sup>

Akihisa Kanamoto<sup>‡‡</sup>

\*Okayama University,

<sup>†</sup>Okayama University,

<sup>‡</sup>Okayama University,

\*\*Okayama University,

<sup>††</sup>Okayama University,

<sup>‡‡</sup>Okayama University,

# Cryptococcus, Pathological observations of five autopsy cases and one biopsy case\*

Katsuo Ogawa, Akira Uejima, Tsuyoshi Inohara, Kiyoshi Kuroda, Joji Murase,  
and Akihisa Kanamoto

## Abstract

Pathologic, anatomical, and histological findings of 5 autopsy cases and one biopsy case of cryptococcosis have been described. Macroscopically the foci of the lung are grayish white or yellowish white in color and range in size from the small acinous-nodular ones to the larger lobular-nodular ones. In the brain the meninx appears gelatinous and edematous showing many small spots with indistinct boundary and with grayish white color. Lymph nodes infected with fungi are swollen in various degrees. Histologically the foci are mainly consisted of granulomatous inflammation containing giant cells. Besides, there are small degenerative foci having no inflammatory response and the lesions of marked fibrosis; the former will be newly formed foci and the latter the old ones. The size of *C. neoformans* found in tissue ranges from 3 to 30  $\mu$ , and the majority of fungi possess thick gelatinous capsule, but some of them in granulative lesions often possess no capsule. From the staining properties the capsule of *C. neoformans* is believed to be a kind of acid mucopolysaccharide. As for the staining method including general fungi, GOMORI's methenamine silver method is best, especially for the detailed examination of fungus structures, and for the differential diagnosis mucicarmin stain is the most suitable one. In tracing the distribution of the foci in the various organs, it seems that the first attack of this fungus occurs in the lung. The authors have called general attention, through their own experiences, to the fact that the small granulomatous foci caused by *Cryptococcus* infection, especially in the lung, may often escape the detection at autopsy.

---

\*Copyright ©OKAYAMA UNIVERSITY MEDICAL SCHOOL

Acta Med. Okayama 13, 319—347 (1959)

**CRYPTOCOCCOSIS**  
**PATHOLOGICAL OBSERVATIONS OF FIVE AUTOPSY**  
**CASES AND ONE BIOPSY CASE**

Katsuo OGAWA, Akira UEJIMA, Tsuyoshi INOHARA  
Kiyoshi KURODA, Joji MURASE and Akihisa KANAMOTO

*Department of Pathology, Okayama University Medical School*  
*Okayama, Japan (Director : Prof. Y. Hamazaki)*

*Received for publication August 5, 1959*

Recently there seems to be an increasing tendency in the incidence of human diseases caused by the infection with various fungi as reported from many countries in the world<sup>1-3</sup>.

This seems to lie in the frequent use of antibiotics<sup>4-12</sup> or in the phenomenon by selection and substitution of microbes—"Sélection et Substitution" de germes sur un terrain en état de moindre résistance (BRISOU)—<sup>13-14</sup>, and also to be due to the advance in examination technics by which the disease becomes easier to be diagnosed<sup>15-16</sup>, as well as to a deeper interest lately shown in such diseases<sup>17</sup>.

Consequently the distribution map on the incidence of fungus diseases as known in the first half of this century has been completely altered.

Likewise in Japan the diseases caused by fungi are increasing annually, especially, an increase in aspergillosis and cryptococcosis has become marked with the comparative decrease of the cases of actinomycosis as is understandable by the report of MIYAKE *et al*<sup>3</sup>. On the other hand, a case of histoplasmosis which hitherto had never been found in this country also has been reported recently<sup>18</sup>.

Of the 814 autopsy cases examined in our department during the past ten years there were 15 autopsy cases of the fungus diseases. Among these 15 cases we found five cases of cryptococcosis including two cases previously reported by MURAKAMI<sup>19</sup> and OGAWA<sup>20</sup>.

In this paper the pathologic findings of these five autopsy cases of cryptococcosis and one additional case detected by the biopsy material are reported in detail with a brief clinical finding in each case.

**CASE REPORTS**

Case 1. 57 years old male, farmer.

Clinical diagnosis : Catarrhal jaundice.

He had jaundice at 30 years old and again at 40 years old. In the middle of November 1951 he complained of general malaise followed by the onset of jaundice. Hospitalized on Jan. 16, 1952. As the results of the physical and laboratory examinations at admission the disturbance of liver functions was recognized with normal body temperature. He had been treated with insulin, glucose, vitamins, Mastigen and glucuronic acid. On Jan. 24, 1952 his body temperature was 37.5°C and he became delirious. Later he gradually fell into coma and in the state of anuria, and expired on Jan. 29.

Main pathological findings: Biliary liver cirrhosis; adenomatous hyperplasia of the choledochus at Papilla Vateri accompanied with advanced mechanical jaundice; catarrhal cholecystitis; dissminated cryptococcosis; biliary parenchymatous degeneration of the kidneys; catarrhal hemorrhagic pneumonia; gastritis phlegmonosa; and congestion of the spleen.

Pathologic findings in detail: The liver was hard and weighed 1,290 g., presenting yellowish green in color. Histologically GLISSON's capsule is thickened with the proliferation of fibrocytes with the infiltration of inflammatory cells. The invasion of connective tissues into the peripheral region of acini can be recognized with the proliferation of false bile ducts. The deposit of bile pigment in liver cells and in stellate cells can be seen with the numerous plugs of the bile.

In the lung, many localized reddish-yellowish white area as big as the tip of thumb were observed scatteringly, mainly located in the subpleural area. Microscopically these are of the granulative inflammation with marked infiltration of lymphocytes, monocytes, macrophages and a few polymorphonuclear leucocytes (Fig. 1). The tissues surrounding these lesions or sometimes lesions themselves present a picture of catarrhal inflammation, revealing the desquamation of epithelium with infiltration of macrophages (Fig. 2) and sometimes with hemorrhage.

In the area where the tissue lesions are severe, small round vacuoles can be seen extra- or intracellularly and within these vacuoles the spheric organisms 3—10 $\mu$  in size and weakly stained with hematoxylin are detected.

Even by the routine fungus stain, the organisms can be identified with fungus having no distinct capsule. A few of them show budding measuring about 20 $\mu$  but there is no hyphal form (Fig. 3). Several mesenteric lymph nodes swollen as large as a pea or a soy-bean present a histologic picture of granulomatous change with the appearance of giant cells. Numerous fungi are found in such area and in the sinuses of the lymph nodes. Other organs such as the spleen, kidneys, heart muscles, adrenals, prostate and the subcapsular region of the liver showed no

macroscopic change, but histologically many small lesions having several fungi or their dense colonies surrounded by the degenerated area without inflammatory change could be observed. Some of these fungi possess a fairly wide space around the body (Figs. 4 and 5). In the kidneys many fungi are found in the markedly-enlarged BOWMAN's capsule (Fig. 6). The brain was not observed.

Case 2. 38 years old house wife.

Clinical diagnosis : Tuberculous meningitis.

No particular history. In Sept. 1953 she had temperature of 37.5°C—38.0°C, accompanied with headache, nausea, and sometimes chill, and she was admitted to the Okayama National Hospital on Sept. 24, 1953.

At admission spinal fluid was watery transparent; 350 mm. H<sub>2</sub>O in initial pressure, after depletion of 12 c.c. the pressure decreased to 130 mm. H<sub>2</sub>O; negative xanthochromia and positive NONNE-APELT's reaction and PANDY's reaction; no fibrin clots; the cell count, 68 cells per cu. mm. (mainly lymphocytes) and the slightly increased sugar content. No other specific findings in various other examinations. In the end of October the body temperature rose to 38°C—38.5°C, with increasing headache and marked systemic emaciation. Spinal fluid proved; positive xanthochromia and fibrin clots, decrease in sugar content, pressure at 250—350 mm. H<sub>2</sub>O, and 90 to 120 cells in per cu. mm. No tubercle bacillus was proved but on the suspicion of tuberculous meningitis dihydrostreptomycin was given from October 23rd to the total dosage of 24.8 g.; 20g. into spinal cavity and 4.8 g. intramuscularly. However, the stiffness of the neck and KERNIG's syndrome grew stronger with increasing headache and emaciation, and she died on November 11, 1953.

Pathological diagnosis : Cryptococcal meningoencephalitis.

Gross findings of the brain; 1, 360 g. Gyri are swollen edematously. Leptomeninges were uniformly lusterless, and many small foci appeared as hazy spots of grayish white color were recognized at the cerebral base, frontal and temporal regions. These foci formed no circumscribed nodules as in the case of tuberculous meningitis but showed indistinct boundary in gelatinous fluid of sulci. The sulci remarkably presented a gelatinous aspect in places. The ventricles were somewhat dilated and contained a large quantity of fluid in high turbidity. The parenchyma, especially at the brain stem, showed a marked hyperaemia with some hemorrhagic lesions. No large cystic lesions could be recognized.

Histological findings : The leptomeningeal cavity is dilated edematously with hyperaemia and a marked and diffuse cell infiltration mainly of lymphocytes with some monocytes and histiocytes (Fig. 7). In this area the localized lesions having irregularly arranged epithelioid cells and foreign body giant cells can be observed (Fig. 8). Extracellularly or intracellularly, especially in giant cells, many organisms faintly stained with hematoxylin could be seen. The organisms are 3 to 15  $\mu$  showing a

great variation in size, and many of them found in giant cells did not show the capsule. But those located in tissue extracellularly had the thick capsule of positive periodic acid-SCHIFF reaction, GOODPASTURE's gram stain showing the characteristics of *Cryptococcus*. The inflammatory changes in leptomeningeal cavity reaches a quite deep area of the brain along the blood vessels (Figs. 9 and 10). In the brain stem some localized little necrotic lesions having fungi in the central parts could be recognized. The fungi are 5—15  $\mu$  in size and have typical capsule (Fig. 11). Other organs than brain were not dissected.

Case 3. 57 years old, a priest.

Clinical diagnosis : Liver cirrhosis.

Had gastric ulcer in 1939 and 1940. In about June 1954 he had felt chill and had the body temperature rise up to 39.4°C. Since the beginning of 1956 he had fever attack once a month and hospitalized in Sept. 1956. The swelling of the liver was pointed out at admission. Discharged from the hospital in Feb. 1957. In May 1957 he had ascites and was diagnosed as tuberculous peritonitis, and treated with Streptomycin. In July 1957 he was admitted to the Clinic of the Okayama University Medical School. Roentgenogram revealed the shadow by tuberculosis in the lung. Examinations suggested the disturbance of the liver function with ascites. From July 13th the body temperature rose to 39°C, and Mycillin and Aureomycin treatments were started. The body temperature reached the normal level after giving the antibiotics and then 1,650 mg. of cortisone in total was given. On about August 23rd the body temperature rose again to 39.2°C and TAKATA's test showed a strong positive. Then Mycillin was given from Sept. 7 to 11 and ACTH gel was given from Sept. 17 to 21, 245 mg. in total. From Sept. 24 again Mycillin was resumed and on Oct. 18, ACTH gel. During this period blood transfusion and depletion of ascites were repeated but the body temperature did not fall and the patient became delirious. On Oct. 25 the patient vomited a large quantity of blood and died on Oct. 26, 1957.

Main pathological findings : LAENNEC's liver cirrhosis with jaundice and ascites, 3,500 c. c.; splenomegaly with chronic congestion weighing 310 g.; chronic granulomatous pneumonia with fibrous thickening of the pleura due to the infection of *Cryptococcus neoformans*; pericarditis fibrinofibrosa; parenchymatous degeneration in kidney, pancreas and liver; and chronic prostatitis.

Gross findings : The liver weighing 520 g. presented a fine granulated surface, and the cut surface also showed the picture of typical atrophic liver cirrhosis forming many small nodules. The lung was fibrously adhered with each thickened parietal pleura, especially marked in the right lung. In some parts just under the thickened pleura hard grayish white masses 0.5—1.5 cm. in diameter could be recognized. They were demarkated with a relatively clear boundary from the adjacent area (Fig. 12). In the brain and other organs macroscopically no lesions considered to be due to the invasion of *Cryptococci* could be recognized.

**Histological findings :** In the liver marked false acini were formed by the proliferation of interstitial connective tissue. In places marked proliferation of false bile ducts in the interstitium can be observed. The lungs show the areas of granulomatous inflammation with the proliferation of fibroblasts and a few giant cells and infiltrated with lymphocytes, monocytes and macrophages. In the central part of these lesions the original lung structure is completely destroyed. In the peripheral area of these lesions the original structures remained, though many mononuclear cells are infiltrated in the alveoli (Fig. 13). In the area surrounding the lesions a slight perifocal inflammation can be recognized. No organisms could at all be detected in the inflammatory center by hematoxylin-eosin stain but GOODPASTURE's GRAM stain revealed a few organisms, GRAM positive. They are positive to GRIDLEY's fungus stain, periodic acid-SCHIFF reaction and GOMORI's methenamine silver stain and found both intra- and extracellularly. They are spherical in shape, measured 3—10 $\mu$ , rarely as big as 15 $\mu$ , and some of large ones have a small single budding. No capsule could be detected by any methods (Fig. 14). The thickened pleura is of the proliferation of connective tissue, with the localized slight cell infiltration, lymphocytes, monocytes and plasma cells. Many colonies of fungi are found here and there in this thickened pleura. The hematoxylin-eosin stain presents a coarse network in the area corresponding to the fungi coloni, reminiscent of a spongy tissue consisted of thinly-walled numerous vacuoles. In this area fungi can be stained faintly with hematoxylin but no noteworthy inflammatory change can be recognized surrounding the colonies (Fig. 15). A large number of fungus cells in the pleural foci are spheric and 10—30 $\mu$  in size. Observation on the frozen section of formol-fixed tissue showed a relatively thick refractive cell membrane on the fungi, whose body is filled with fine granular substances. Within the protoplasm always one, sometimes 2 or 3 homogeneous nucleus-like droplets 1—3 $\mu$  in size are visible. By treating with Sudan III, these droplets are stained orange-red (Fig. 16). Some fungi show a small nodular or short tubular single budding (the germ tube), as can be seen in paraffin section.

These fungus cells are surrounded with some gelatinous capsule as thick as about 10 $\mu$ . The presence of the capsule can be clearly detected in the frozen section added with a droplet of India ink (Fig. 17) or by GRAM stain, mucicarmine or periodic acid-SCHIFF reaction. The fungi without capsule can also be found in the connective tissue. Even such organisms are also far bigger than those found in the lung parenchyma and 15—20 $\mu$  in size.

That is to say in the lesions of the lung parenchyma and the pleura of this case, concerning the capsule and size two different types of fungi are found at the same time.

In the brain, lymph node and even in the cirrhotic liver tissue the foci considered to be due to the infection of *Cryptococcus* can not be found.

Case 4. 28 years old, male, farmer.

Clinical diagnosis : Acute lymphatic leukemia.

In about the middle of August 1957 he had fever and severe anemia without any apparent cause. Blood test revealed the increased leucocyte count, 300,000/mm.<sup>3</sup> and admitted to the Clinic of the Okayama University Medical School. By the morphologic examinations of blood cells both on smears and bone-marrow tissue culture, he was diagnosed as acute lymphatic leukemia. At admission Hb-content was 34 per cent SAHLI, the erythrocyte count 2.29 million, the leucocyte count 203,800/mm.<sup>3</sup>, color index 0.74, lymphocytes and lymphoblasts occupied 87.2 per cent of the whole white blood corpuscles. After admission remission had been repeated in the course of treatment with prednisolone and 6 M.P. etc., and lymphoblasts disappeared in the circulating blood for a time being. From the end of 1957 again the lymphoblast count and the bleeding tendency increased with worsening in general conditions. The blood test conducted in Jan. 1958 showed; Hb-content 28 per cent SAHLI, the erythrocyte count 1.51 million, the leucocyte count 47,500/mm.<sup>3</sup> and 94.4 per cent lymphoblasts. From about Feb. 5th the excited state continued and gradually the heart sound grew weaker, and the patient died on Feb. 7, 1958.

Main pathological findings ; Acute lymphatic leukemia with the leukemic cell infiltration in various organs; i. e. lymphnodes, liver (weighing 2,625 g.), spleen (1,330 g.), kidneys, bone marrows, heart muscle, lungs, pancreas, testes and leptomeningeal cavity. Hemorrhagic diathesis; and Cryptococcosis of the right lung.

Gross and histologic findings: In the organs of the entire body marked changes due to leukemia can be recognized. In the lungs, aside from the lesion by leukemia, a marked catarrhal pneumonia due to bacterial infection could be recognized. In the middle part of the right lung, one grayish white semitransparent localized area with a gelatinous aspect as big as the tip of thumb (Fig. 18) was found. Histologically this part is of the complete coagulation necrosis surrounded by granulative tissue with marked proliferation of fibrocytes accompanied with the infiltration of lymphocytes, a few histiocytes and giant cells (Fig. 19).

Elastic fiber staining revealed the original alveolar structure remained in the necrotic area (Fig. 20). The necrotic region has many spherical vacuoles which contain round fungi, measuring 15—30 $\mu$ , and possessing a capsule faintly or sometimes deeply stained with hematoxylin. The presence of the capsule about 10 $\mu$  thick had been clearly verified by mucicarmine, GRAM, GRIDLEY's and other fungus stains (Fig. 21). A few of



them had no capsule (Fig. 22). Some of them showed an indentation on one side of cell membrane as if pressed from outside and some others have budding (Fig. 23).

Case 5. 38 years old female, unoccupied.

Clinical diagnosis : Uremia due to chronic nephritis.

She was suffering from high blood pressure and in August 1958 she received the treatment for hypertension. In September she was told to have disturbances in the kidneys, liver and heart at the Osaka National Hospital. Soon after she had edema and on October 20, 1958 she was admitted to the Okayama Saiseikai Hospital. Findings at admission were ascites retention, the swellings of liver and spleen, edema in lower ribs, the enlarged heart, and high blood pressures, 245/135 mm. Hg. Urine examinations revealed a marked protein excretion, 4.2% ESBACH, and erythrocytes, leucocytes and desquamated epitheliums of the kidneys in moderate number. Blood examinations showed the decreased erythrocyte count, 3.22 mil., and the nonprotein nitrogen, 40.6 mg./dl.

On December 16, 1958 the erythrocyte count was 1.80 mil., and the nonprotein nitrogen, 81.5 mg./dl. Thereafter, edema and ascites increased with the increased amount of nonprotein nitrogen. The somatic conditions grew worse and worse. By May 1959 the surface of the swollen liver began to present granulation. The spleen became palpable, four digits width below the costal margin. Test on liver function revealed negative and sometimes positive TAKATA's reaction and GROSS's reaction, C. C. F. was negative but sometimes strong positive. She died on May 14, 1959.

Main pathological findings : Secondary nephrosclerosis accompanied with uremia ; liver cirrhosis of LAENNEC's type ; hypertrophy of the heart muscle with small necrotic lesions due to infarction ; ascites, 2,800 c.c., hydropericarditis, fluid 440 c.c. ; chronic congestion and fibrosis of the lungs ; cryptococcosis of the left lung ; and atherosclerosis.

Histologically the kidney has almost no normal MALPIGHIAN corpuscles with a greatly devastated parenchym, presenting a picture of typical nephrosclerosis. The liver shows the picture of typical annular fibrosis with the formation of pseudo-acini. The normal structure of liver is severely distorted and individual cell shows fairly strong degeneration.

The lungs macroscopically show the chronic congestion picture with fairly strong fibrosis. Several localized foci somewhat hard and as big as a millet with grayish white color whose center is yellow, can be observed on the subpleural region in the upper lobe of the left lung (Fig. 24). Microscopically in these foci the alveolar spaces have proliferated fibroblasts, presenting a picture like the cornification in pneumonia but the alveolar septa still retain more or less their own original structure. Also the infiltration of lymphocytes, a few monocytes and giant cells can be seen in these foci (Fig. 25). The central parts of the focus present the picture of necrobiosis with a marked infiltration of lymphocytes and polymorpho-nuclear leucocytes and the fungi can be seen either ingested in

the macrophages or free out of cells, which are as big as 15—20 $\mu$  and stained faintly with hematoxylin (Fig. 26). They are spheric and have the thick cell membrane stained relatively deep and present a picture as if the cell itself is a vacuole with lightly stained contents. They have no budding nor typical gelatinous capsule (Fig. 27), though a few of them show indistinguishably-thin capsule around the cell membrane.

**Mycological findings:** Fungus culture was performed using the damaged tissue in the lung at autopsy. A few days' culture on SABOURAUD's glucose agar medium demonstrated the colonies having milky white wet and smooth surface. Microscopical observation revealed that the colonies are of fungi having thick cell-membrane, spheric in shape and 5-15 $\mu$  in size. A few of them showed a single budding. Gelatinous thick capsule could not be recognized around the organisms, though a few of them had an atypical thin capsule (Fig. 28). With respect to the capsule these findings coincide with that of fungi in tissue.

They have glycolytic ability as shown in Table 1, and can be distinguished from the *Candida* species. Namely, concerning the utilization of sugar the fungi require no lactose, but galactose, maltose, saccharose, glucose and fructose as shown in Table 2, and the extracellular production of starch-like compound can be recognized in the synthetic dextrose-thiamine-agar medium.

By observations the organism is identified as *Cryptococcus neoformans*.

Table 1 Glycolytic ability of the isolated fungus

Days	Lactose	Maltose	Glucose	Saccharose
	Acid/Gas	Acid/Gas	Acid/Gas	Acid/Gas
1	-/-	?/-	?/-	-/-
2	-/-	?/-	+/-	?/-
6	-/-	?/-	+/-	+/-
18	-/-	+/-	+/-	+/-
21	-/-	+/-	+/-	+/-

Table 2 The utilization of sugar

Sugars Days	Lactose	Galactose	Maltose	Saccharose	Glucose	Fructose
3	-	-	-	+	+	+
5	-	+	+	+	+	+

**Case 6. 46 years old female, farmer.****Clinical diagnosis: Fungus disease.**

At the mass physical examinations held in May 1958 she was diagnosed as pulmonary tuberculosis and after about a month's treatment she improved.

In November 1958 she complained of the swelling and pain in various joints with fever of about 39°C. accompanied with chill. A little later the troubles of joints were alleviated, but the swelling of somatic lymph nodes appeared. Lymph nodes gradually grew in size, some reaching the size as big as a walnut but the majority of them about the size of a horse bean, being not so hard. Physical examination proved the swollen liver palpable at three digits-width below costal margin and solid. Both palatine tonsils were grayish white with rugged surface. The spleen was not palpable but the area of splenic dullness was extended. The thoracic roentgenogram suggested a fibrous tuberculous focus in the left lung. Blood cell count revealed the increased leucocyte number, 45,000/mm.<sup>3</sup> with the increase in atypical lymphocytes occupying 70 per cent of the leucocytes, and a few fungi could be detected in peripheral blood smears (Fig. 29). The culture of blood gave a negative result provably due to the faulty technic. The culture of cerebrospinal fluid, lymph nodes, and urine presented the colonies of *C. neoformans* and Mycostatin treatment was commenced on April 16th and her body temperature fell for a time being. But on about April 25th her consciousness became hazy, and she died on April 30th. Autopsy was not performed on this case.

**Pathological findings of the biopsied axillary lymph node:** The lymphnode was as big as a walnut, grayish white in color, but not so hard.

Microscopically the original structure of lymph nodes is completely lost by the diffuse infiltration of atypical lymphocytes and lymphoblasts with the irregular proliferation of reticulum cells, the picture is reminiscent of the lymph nodes in leukemoid reaction. In a portion of the swollen lymph node reticulum cells are proliferated forming many giant cells, which often contain large vacuoles in their protoplasm. In the vacuoles spherical fungi 3—10 $\mu$  in size can be recognized. They have thick capsules that is stained especially deep with mucicarmine (Fig. 30). Fixation with CARNOY's fluid gives more distinct picture of capsules than that in formalin fixation. However, there are some small fungi having no capsule. In this case the inflammatory cell infiltration is not recognized in the focuses or around them.

**Histological diagnosis:** Cryptococcal infection in the axillary lymph node accompanied with leukemoid reaction.

**DISCUSSION**

In all six cases presented cryptococcosis occurred in the prime age of life as can be seen in Table 3. This coincides with the age range on the occurrence of fungus diseases as reported by MIYAKE<sup>17</sup> in his autopsy study on the fungus diseases in Japan.

Table 3 Pathological and mycological findings

Case No.	Age & sex	Pathological diagnosis	Organs involved			Form of fungus						Culture	
			Without infl. reaction	Granulative inflammation	Ne-crotic	With capsule			Without capsule			Clinical	Autopsy
						Large	Small	Not uniform	Large	Small	Not uniform		
1 (1201)	57, M.	Biliary liver cirrhosis	Kidneys, heart, liver, spleen, adrenals and prostate	Lungs and lymphonodes			In every lesions				Lungs & lymph nodes	/	/
2 (56) (National Hospit.)	38, F.	Cryptococcal meningo-encephalitis		Meninx & brain	Brain			Meninx & brain			Meninx	-	/
3 (1691)	57, M.	Laennec's liver cirrhosis	Pleura (like scar tissue)	Lungs		Pleura					Lungs	/	/
4 (1731)	28, F.	Lymphatic leukemia			Lung	Lung			Lung			/	/
5 (1875)	38, F.	Nephrosclerosis & Laennec's liver cirrhosis		Lung							Lung	/	+
6 (Biopsy)	46, F.	Disseminated cryptococcosis accompanied with leukemoid reaction		Lymph node			Lymph node					+	/

In our observation, as can be understood from Table 4, five cryptococcoses out of 15 total cases of mycoses are extremely high in the frequency comparing with the MIYAKE's report<sup>17</sup> in which 24 cryptococcoses were found out of 193 mycosis cases in the past ten years, a statistical observation in Japan. Because of the paucity in the number of the cases studied, it requires further research to know definitely whether or not there are specific geographical conditions in Okayama Prefecture for this disease, but it is expected that there are fairly a great difference in the occurrence frequency in each district. In contrast the incidence of true candidiasis encountered in our laboratory is rather low in percentage comparing with that of MIYAKE's report. *Candida* frequently attacks the upper alimentary canal such as in the oral cavity or in the esophagus, and clinically it is easily discovered and often cured completely. Possibly for this reason, as stated by KOBAYASHI<sup>21</sup>, HAMAZAKI *et al.*<sup>22</sup> it is supposed that there are many cases in which the *Candida* was detected during the life time and cured and subsequently found not at all at autopsy. In some cases demonstrating suspicions and revealing the organisms even in culture of the autopsy material, the organisms can hardly be detectable histologically. *Candida* is frequently detected at the upper respiratory tract or at the digestive canal showing no pathogenicity, but no *Cryptococcus neoformans* can usually be found in such portions. In fact RIETH<sup>23</sup> attempted the isolation of various fungi from epidermis, respiratory tracts and other parts of human body and reported that there was no single case that he could isolate *Cryptococcus neoformans* from these parts. EMMONS<sup>24</sup>, on the other hand, successfully isolated a pathogenic form of *Cryptococcus neoformans* from soil by passage through mice. These facts seem to indicate that the infection by *Cryptococcus neoformans* is clearly of exogenous origin and the fungi can not stay in human body without giving any lesion. They will invade into the lung by way of the upper respiratory tract and the lesion in the lung is established for the first time in the human body<sup>25,26</sup>. Ever since SHEPPE<sup>27</sup> first reported in 1924 on the primary lesions in the lung caused by the infection of *Cryptococcus neoformans*, there appeared many reports on the lesions in lung by many investigators such as HAUGEN and BAKER<sup>28</sup> and by others<sup>29,30,31,32</sup>. However, GENDEL *et al.*<sup>33</sup> state that the meningoencephalitis by *Cryptococcus* complicated with the lung lesion amounted to 20 per cent of the cases they studied and the infection occurring in the lung alone was still rarer. But in the cases having small lesions in the lung, there is a great possibility that it often escapes its detection at autopsy. HAUGEN and BAKER<sup>28</sup> are of the opinion that a careful observation may increase the discovery of the

primary lesions in the lungs. The authors fully agree to their opinion through the experience on the above mentioned cases, especially on the cases 3, 4, and 5.

Table 4 Incidence of the fungus infection observed in our laboratory during the last ten years.

Diseases	Numbers of cases	
Cryptococcosis	5	15
Candidiasis	6	
Actinomycosis	2	
Candidiasis + Aspergillosis	1	
Histoplasmosis	1	
Total of autopsy cases	814	

Regarding the cause of the rapid increase of mycosis in recent years opinions are different among several authors<sup>4-17</sup>.

Most of the investigators attribute the cause to the use of modern therapeutic agents such as antibiotic drugs, hormones and marrow depressants. However, some others believe that there are other problems yet to be solved on this contention<sup>14,34</sup>. ZIMMERMAN<sup>10</sup> summarizes the causes to these main factors; (a) an underlying state of poor resistance; (b) a locus minoris resistentia; and (c) ecologic disturbance, often brought about by antibiotics, cortisone or ACTH.

As shown in Table 5 four cases studied by the authors received modern therapeutics such as antibiotics or hormones etc. and it is suspected that the use of such drugs worsened symptoms in Case 2 as can be understood from her clinical investigations, but in other cases the cryptococcal infection developed without use of antibiotics etc. suggesting that the disease can develop without any aid of such drugs.

However, the clinical and autopsy findings on Cases 2 and 6 show that *Cryptococcus* infection is certainly the fatal cause in these cases, whereas in all deaths by some other causes the infection was found incidentally at autopsy. This suggests that there are specific conditions including the treatment with antibiotics, at which the organism is susceptible to *Cryptococcus* infection.

There are some case reports of cryptococcosis accompanied with liver cirrhosis in our country<sup>35,36</sup>. As shown in Table 3 we also encountered three cases of cryptococcosis that had liver cirrhosis as the preexisting disease. In the cases of candidiasis, too, we encountered two cases having liver cirrhosis<sup>22,35</sup> out of 6 cases met with in our department. In the cases

Table 5 Therapeutics used

Case No.	Clinical diagnosis	Therapeutics
1	Catarrhal jaundice	Insulin, Glucronic acid and Vitamins
2	Tuberculous meningitis	C. M.      S. M.
3	Tuberculous peritonitis and liver cirrhosis	S. M., A. M., Mycillin, ACTH and Cortisone
4	Acute lymphatic leukemia	Prednisolone and 6 M. P.
5	Chronic nephritis and uremia	Neophirin, heart medicine and Chlortoride
6	Cryptococcosis	INAH, T. M., ACTH and Mycostatine (3,900 unit)

Notes : C. M.: Chloromycetin, S. M.: Streptomycin,  
A. M.: Aureomycin, T. M.: Terramycin,  
INAH: Isonicotinic acid hydrazide.

having liver cirrhosis no fungus was detected in the cirrhotic liver tissue excepting only one case of cryptococcosis, therefore it can be said reasonably that liver cirrhosis acts as to increase the susceptibility of the organisms to the fungi.

The complication of fungus disease with the diseases of hematopoietic organs appear in many reports<sup>11, 33, 37, 38, 39, 40, 41</sup> and we encountered two cases, namely, Cases 4 and 6. In the Case 4, *Cryptococcus* infection occurred in the lung infiltrated with leukemic cells. On the other hand, in the Case 6 the cell response in the lymph node seems to be due to disseminated cryptococcosis and the leukemia-like picture will probably be of leukemoid reaction caused by the fungus infection. In such an instance there are some reports<sup>38, 39</sup> on the case in which *Cryptococcus* infection induced the leukemoid reaction. These findings suggest that the development of the disease is closely correlated to the disturbance of hematopoietic organs. With respect to the disseminated infection of *Cryptococcus* combined with the malignant disease of the reticuloendothelial system, LITTMAN and ZIMMERMAN<sup>26</sup> state that this fungus cannot be construed as the cause for malignant disease of this system, and ZIMMERMAN<sup>40</sup> claims reasonably that the administration of drugs such as marrow depressants is the foremost cause bringing about the low resistance in the reticuloendothelial system and thus the *Cryptococcus* infection ensues.

Concerning the distribution of the cryptococcal foci in our case, it is shown in Table 3 showing the highest incidence in lung. The case with the lesions limited solely in the brain as mentioned in the textbooks<sup>25, 42</sup> or in the general reports<sup>43, 44</sup> we encountered only a single case (No. 2) among

6 cases. In Case 1 showing disseminated cryptococcosis, findings reveal that the lesions had primarily ensued in the lung and later disseminated to other organs. Therefore, even in Case 2 where the lesions could be observed in the brains, there is every possibility that the primary lesions might have existed in the lung, had other organs been dissected. FREEMAN<sup>49</sup> divides the lesions in the brain into 3 types: 1) a meningeal type which is most common and is characterized by a diffuse granulomatous meningitis; 2) a perivascular form accompanied by small granulations or cysts in the cortex; and 3) an embolic type deeply placed lesion in the cortex. In our Case No. 2 the lesions of brain presented all the three types and the finding of the type 1 appeared most prominently. About the lesion in the brain parenchyma it is said that the lesions most frequently occupy the position of VIRCHOW-ROBIN's space<sup>46</sup> and that their granulation tissue producing the argyrophil fibers is the characteristic trait<sup>3</sup>; these findings were observed in our case, too, but the proliferation of monsterglia reported by ZEMAN<sup>50</sup> could not be found.

The lesions in the lung are practically all located in pleura or subpleural region but no cryptococcal infection in the apex has been found not in a single case differently from the case of tuberculosis in which primary lesions are frequently distributed in the upper lobes of the lung. Actually, the lesions in Cases 1 and 3 were mainly distributed in the middle and lower lobes and in Case 4 the lesion was in *Segmentum superius*. Case 5 had the small lesions limited in the upper lobe but they were distributed far away from the apex and in the lateral part of the lung near the region where pleura transposes to *Fissura interlobaris*. Individual focus usually occupied the area of a single lobule as a unit and smaller ones take the picture of acinous- nodular lesions. These small lesions in the lung parenchyma may sometimes regress and disappear, but usually it seems to fuse with adjacent lesions as seen in Case 5 and gradually grow larger, forming subpleural lobular lesions as in the other cases.

Three cases 1, 3 and 4, having pulmonary lesions presented adhesive fibrous pleurisy with the thickened pleura as mentioned already and in Case 3 many fungi were detected in the pleura tissue. But in our experience met with Case 3 having a comparatively severe congestion of the lung at the first of autopsy, we failed to detect the subpleural lesions. Therefore, the observation must be conducted most carefully to detect the small foci.

Next, the pathohistologic findings will be discussed in detail. Generally yellowish white but red patches, combining with bleeding, appear as in



Case 1. Marked bleeding gives a picture like hemorrhagic bronchopneumonia. When the proliferation of fungi is marked, the foci give a semi-transparent gelatinous appearance because of their gelatinous capsule. These lesions are generally demarkated from adjacent area by the relatively distinct boundary, especially marked in the absence of perifocal inflammation.

The characteristic morphologic feature of this disease is usually a chronic granulatory inflammation but it is hard to point out the specificity representing this disease.

BAKER<sup>45</sup>, OKUDAIRA<sup>46</sup> and HAMAZAKI<sup>22</sup> pointed out a wide variety of histological structures shown by individual lesions.

In our observation, in dividing these lesions roughly, they fall into two categories; namely, the ones that show no inflammatory cell reaction and the other, that have strong cellular responses. Usually in the former many fungi possessing gelatinous capsule are congregated in the cyst-like structure. These lesions are thought to be<sup>36,47</sup> newly formed foci by the terminal dissemination occurring just before death, as in Case 1. Whereas the latter are accompanied by the marked inflammatory reaction with mononuclear cell infiltrations but almost no infiltration of polymorpho-nuclear leucocytes, as pointed out by CONANT *et al.*<sup>23</sup>, BAKER<sup>45</sup>, etc.

In the relatively early stage the lesion is mainly infiltrated with lymphocytes with some monocytes and macrophages, and in a few instances a few plasma cells or eosinophils. In the case where the lesion shows necrosis in its center, sometimes a few polymorpho-nuclear leucocytes can be observed (Fig. 26).

Furthermore, in the stadium of far advanced stage fibroblasts will conduct the inflammation sometimes being arranged in the type of epithelioid cells. By this stadium, many giant cells appear. They are of foreign body giant cell type, rarely of LANGHANS' type, which contain always fungi. In the area where fibroblasts increase with the formation of fibrous lesion, lymphocytes gradually decrease. Some focus seems to turn to necrosis instead of progressing towards fibrosis. In such an instance the majority show necrosis in the center of focus and present somewhat a tubercle-like structure. However, the cells are not orderly arranged as in tuberculosis but it is in a rather disorderly mixture of cells with many fungi among them.

Sometimes tissue reaction takes place only by the proliferation of indigenous cells of the organ; i. e. in the lungs the alveolar spaces are filled with desquamated alveolar epithelium without inflammatory cell infiltration and many fungi can be detected extracellularly or intracellularly,

sometimes presenting a picture of desquamative pneumonia (Case 1, Fig. 2). The lesions of lymph nodes in the patient with lymphatic leukemoid reaction (Case 6) showed diffuse proliferation of reticulum cells, presenting an appearance of markedly swollen foam cells. Within these cells many fungi could be found but no infiltration of inflammatory cells in the same region.

The case in which the lesions of the lung show extensive coagulation necrosis are extremely rare as compared with the cases of lung tuberculosis, but occasionally such cases are reported (SHIMOMURA<sup>48</sup>, BAKER<sup>45</sup>). Almost all the lung lesions of Case 4 also fall into the coagulation necrosis, and around these necrotic foci only a small amount of the granulomatous tissue composed of the well-retained alveolar structure can be detected by the elastic fiber stain. Therefore, it seems that prominent exudative inflammation must have occurred at first.

As for the calcification there can be recognized not a single case in our experiences.

Many investigators<sup>51,52,53</sup> reported on the variation of human pathogenic fungi that not only the patterns of tissue response but also that of fungi themselves found in tissues are multifarious. Namely, in the case of *Candida* or *Aspergillus* that takes two forms of yeast and hyphal; and *Histoplasma* or *Blastomyces* takes commonly round shape in tissue, but both of these fungi present a variety in size and are divided into small and large or giant forms.

*Cryptococcus* also takes various patterns. The smaller ones measure 3–10 $\mu$  and larger ones reach the size as big as 30 $\mu$ . These fungi may possess characteristic gelatinous capsule while others may not, showing a wide variety of the morphological patterns in tissues. The capsule is consisted of transparent gelatinous substance stained faintly but sometimes deeply with hematoxylin (Fig. 31), but it is negative to fat-stain. It is positive to mucicarmine<sup>54</sup> or to polysaccharide stain while to thionin stain it presents metachromasia. Therefore, it is assumed that the capsule is composed of a kind of acid mucopolysaccharides, which KLIGMAN<sup>55</sup> calls as hyaluronic acid.

KASUGA *et al.*<sup>38</sup> state that the capsule can be stained with mucicarmine in a beautiful radial form, while LITTMAN and ZIMMERMAN<sup>26</sup> present a similar picture of frozen section of the capsule stained with toluidine blue in photograph. However, this may be only an artifact observable in formalin fixation (Fig. 32); namely, when the capsule is treated with some other fixatives containing no water such as CARNOY's fixative, it is stained as a homogeneous substance (Fig. 33). And also nucleus-like small globules

found in cytoplasm are stained well with Sudan III (Fig. 16) as mentioned by LITTMAN and ZIMMERMAN<sup>26</sup> and also by MIYAKE *et al.*<sup>3</sup>

Some of relatively large organisms show a single budding, and this budding sometimes extends still further, forming germ tube (Fig. 23).

Generally in the lesions not accompanied with inflammatory reaction organisms as big as  $10\mu$  with the capsule can often be found. In the older lesions showing strong necrosis or fibrosis many large organisms  $20-30\mu$  in size can be found and most of them have extremely thick capsule.

In the lesions revealing marked cellular response the size of fungi ranges variously from 3 to  $15\mu$ , and often fungi without capsule can be detected extra- or intracellularly. Sometimes the capsule can be detected even around those organisms found within giant cells (Fig. 34).

Decisive diagnosis is, of course, possible only when the fungi having thick gelatinous capsule are detected. However, the fungus body is not always easily found in the foci of the granulomatous inflammation by hematoxylin-eosin staining. Though in rare instances, fungi can be stained faintly with hematoxylin, in the most frequently-observable granulomatous foci they can not be usually detected by this method as stated by CONANT *et al.*<sup>25</sup>

There are many reports<sup>56,57,58,59</sup> on the evaluation of staining technics of fungi. We employed the routine fungus stains that are in common use; namely, GOODPASTURE's GRAM stain, GRAM-WEIGERT's, periodic acid-SCHIFF reaction, LILLIE's alochrome<sup>60</sup>, GRIDLEY's fungus stain<sup>61</sup>, BAUER's, BIELSCHOWSKY's silver and GOMORI's methenamine silver methods<sup>62</sup>. GRAM stain does not yield much good result for the detection of fungi in granulative inflammatory tissue. As a rule these fungi are positive to the GRAM stain, but sometimes the fungus body or the capsule is gram-negative (Fig. 35). By periodic acid-SCHIFF reaction and LILLIE's stain it is sometimes difficult to differentiate small fungi from other cellular substances that are stained deep. Of these two methods from the standpoint of contrast LILLIE's stain is superior. By GRAM, mucicarmine stain or periodic acid-SCHIFF reaction the capsule can be stained, but by GRIDLEY's or BAUER's stain the capsule can hardly be stained. However, with the latter two methods because only little other cellular substances are stained, they can be recommended as useful methods for the fungus detection.

GOMORI's method is not only the most suitable one for *Cryptococcus* but also for general fungi because it makes the internal structures of a fungus distinct and also it gives a better contrast with other tissue components as well as affords a better detection of fungi.

For the staining methods of *C. neoformans* with a special reference to

the specific gelatinous capsule, we employed mucicarmine stain, metachromasia by thionin stain, as well as Indian-ink methods as the detection method. By applying droplets of Indian-ink it is possible to detect readily the capsule as a transparent layer around the fungus body (Fig. 17). LITTMAN and ZIMMERMAN<sup>26</sup> state that RHINEHART-ABUL-HAJ technic for acid mucopolysaccharides for the detection of mucin in the capsule and the cell wall of *C. neoformans* will play the most important role in the differential diagnosis of this fungus. Aside from the above mentioned, MIYAKE et al.<sup>58</sup> claim that JÄRVI-LEVANTO's silver method is suitable for the general fungus stain.

The only one in which fungi were detected during the patient's life time by the culture is Case 6, but no detailed examination was conducted on this case. And the only one in which fungi were detected from autopsy material by the culture is Case 5. The characteristic of this fungus colony on SABOURAUD's medium is a creamy feature of its smooth surface. The fungus in culture is of a spheric shape and 3–10  $\mu$  in size with thick refractive cell membrane. Only a few of them possess a thin capsule but the majority do not have capsule, and MIKAMO<sup>63</sup> also observed variation of fungi without capsule (Fig. 28).

As BAKER and HAUGEN<sup>45</sup> mentioned, the morphological findings of the fungi in tissue of our cases coincide with those of fungi in culture. As for the physiologic properties of the fungi in culture, the fungi produce acids by decomposing sugar but emit no gas; and this point agrees with the finding of LITTMAN<sup>26</sup>.

#### SUMMARY

Pathologic, anatomical, and histological findings of 5 autopsy cases and one biopsy case of cryptococcosis have been described.

Macroscopically the foci of the lung are grayish white or yellowish white in color and range in size from the small acinous-nodular ones to the larger lobular-nodular ones. In the brain the meninx appears gelatinous and edematous showing many small spots with indistinct boundary and with grayish white color. Lymph nodes infected with fungi are swollen in various degrees.

Histologically the foci are mainly consisted of granulomatous inflammation containing giant cells. Besides, there are small degenerative foci having no inflammatory response and the lesions of marked fibrosis; the former will be newly formed foci and the latter the old ones.

The size of *C. neoformans* found in tissue ranges from 3 to 30  $\mu$ , and

the majority of fungi possess thick gelatinous capsule, but some of them in granulative lesions often possess no capsule. From the staining properties the capsule of *C. neoformans* is believed to be a kind of acid mucopolysaccharide.

As for the staining method including general fungi, GOMORI's methenamine silver method is best, especially for the detailed examination of fungus structures, and for the differential diagnosis mucicarmine stain is the most suitable one.

In tracing the distribution of the foci in the various organs, it seems that the first attack of this fungus occurs in the lung. The authors have called general attention, through their own experiences, to the fact that the small granulomatous foci caused by *Cryptococcus* infection, especially in the lung, may often escape the detection at autopsy.

#### ACKNOWLEDGEMENT

We wish to express our profound thanks to Prof. Y. HAMAZAKI, Department of Pathology, Okayama University Medical School, for his invaluable advices and to Dr. H. YAMATO, Director of Saiseikai Hospital, Okayama City, for his assistance in our work. We are also greatly indebted to Dr. Chester W. Emmons of National Institute of Health and to Dr. Chapman Binford and Dr. Lorenz E. Zimmerman, the Armed Forces Institute of Pathology, U.S.A., for their kind suggestions and encouragement.

#### REFERENCES

1. OKUDAIRA, M. : A statistical and histopathological study of mycotic infections. *Acta Path. Jap.* 6, 207, 1956
2. KEYE, J.D. & MAGEE, W.D. : Fungal disease in a general hospital. A study of 88 patients. *Am. J. Clin. Path.* 26, 1253, 1956
3. MIYAKE, M. & OKUDAIRA, M. : Pathological studies on the fungus diseases. *Saishin-Igaku.* 13, 870, 1958 (in Japanese)
4. MIKAMO, Y. : Antibiotics and monilia. *Nihon-Rinsho*, 10, 105, 1952 (in Japanese)
5. OKINAKA, S., KUBO, I. & TOGO, Y. : Antibiotics and Mycosis. *Jap. J. Clin. Path.* 2, 196, 1954 (in Japanese)
6. WOODS, J.W., MANNING, I.H. & PATTERSON, C.N. : Monilial infections complicating the therapeutic use of antibiotics. *J. A. M. A.*, 145, 207, 1951
7. MIYAKE, M. & OKUDAIRA, M. : Pathology of the fungus diseases. *Kin-kotaisho*, (Affections par microbiosme sélectionné et substitué) *Igakushoin*. Tokyo, Osaka, 1958 (in Japanese)
8. WYBEL, R.E. : Mycosis of cervical spinal cord following intrathecal pencillin therapy. *Arch. Path.*, 53, 167, 1952
9. ZIMMERMAN, L.E. : Candida and aspergillus endocarditis. *Arch. Path.* 50, 591, 1950
10. ZIMMERMAN, L.E. : Fatal fungus infection complicating other disease. *Am. J. Clin. Path.* 25, 46, 1955
11. CRAIG, J.M., and FARBER, S. : The development of disseminated visceral mycosis

- during therapy for acute leukemia. Am. J. Path. 29, 601, 1953
12. BEUTHE, D. : Candidasepsis. Zbt. f. allg. Path. 93, 241, 1955
13. BRISOU, J. : Affections chroniques par microbiosme sélectionné et substitué. Presse Med. 60 : 353, 1952
14. OKUDAIRA, M. : Moniliasis, recent advance in fundamental medicine (II). 283, Ishiyaku Publication Co., Ltd, Tokyo, 1958 (in Japanese)
15. STARR, G. F. : Use of periodic acid-Schiff in identification of pathologic fungi in tissue. Am. J. Clin. Path. 55, 76, 1955
16. ZIMMERMAN, L. C. L. : Demonstration of *Histoplasma* and *Coccidioides* in so-called tuberculomas of lung. Arch. Int. Med. 94, 690, 1954
17. MIYAKE, M., OKUDAIRA, M., and SETO, T. : Report by the Mycosis Research Corps. at Tokyo University. 1958
18. YAMATO, H., HITOMI, H., MAEKAWA, S., MIMURA, K., HAMAZAKI, Y., OGAWA, K., & UEJIMA, A. : A case of histoplasmosis. Report I. Clinilcal, mycological and pathological observations. Acta Med. Okayama 11, 347, 1957
19. MURAKAMI, I. : A case of "disseminated torulosis". Okayama-Igakkai-Zasshi 65, 624, 1953 (in Japanese)
20. OGAWA, K. and MATSUMOTO, M. : On an autopsy case of *Torula meningoencephalitis*. J. Karyopathology 1, 410, 1954 (in Japanese)
21. KOBAYASHI, T. : Pathology of the moniliasis. Gendai Igaku 3, 280, 1954. (in Japanese)
22. HAMAZAKI, Y., OGAWA, K., HAMAZAKI, M., ARIKI, I., UEJIMA, A., and YONEDA, S. : Case studies on the fungus infection (encountered in our laboratory). Acta Path. Jap. (in the press)
23. RIETH, H. : Untersuchung zur Hefe-diagnostik in der Dermatologie. Arch. klin. und exper. Dermat. 207, 413, 1958
24. EMMONS, C. W. : Isolation of *Cryptococcus neoformans* from soil. J. Bact. 62, 685, 1951
25. CONANT *et al.* : Manual of clinical mycology. W. S. Saunders Co., 1954
26. LITTMAN, M. L. and ZIMMERMAN, L. E. : Cryptococcosis. Grune & Stratton, New York & London, 1956
27. SHEPPE, W. M. : *Torula* infection in man. Am. J. M. Sc. 167, 91, 1924
28. HAUGEN, R. K. & BAKER, R. D. : The pulmonary lesion in cryptococcosis with special reference to subpleural nodules. Am. J. Clin. Path. 24, 1381, 1954
29. HAMILTON, J. B. & TYLER, G. R. : Pulmonary torulosis. Radiology 47, 149, 1946
30. GREENING, R. R. & MENVILLE, L. J. : Roentgen findings in torulosis. Report of four cases. Radiology 48, 388, 1947
31. TERPLAN K. : Pathogenesis of cryptococcic (*Torula*) meningitis. Am. J. Path. 24, 741, 1948
32. FORBUS, W. D. : Quoted from Zimmerman (40)
33. GENDEL, B. R., ENDE, M. & NORMAN, S. L. : Cryptococcosis, a review with special reference to apparent association with Hodgkin's disease. Am. J. Med. 9, 343, 1950
34. KLIGMAN, A. M. : Are fungus infections increasing as a result of antibiotic therapy? J. A. M. A. 149, 979, 1952
35. HAMAZAKI, M. & HASHIDA, T. : Systemic candidiasis with involvement of central nervous system. Report of a case. J. Karyopathology 2, 141, 1954 (in Japanese)
36. Inui, M., OKUDA, T., and MATSUMOTO, K. : Two autopsy cases of mycotic meningoencephalitis. Tr. Soc. Path. Jap. 47, 402, 1958 (in Japanese)
37. KAWAJI, K. & HASHIGUCHI, T. : Three cases of the Histoplasmosis? or Blastomycosis? Tr. Soc. Path. Jap. 43, 459, 1954 (in Japanese)

38. KASUGA, T. & YOSHIMITSU, E. : An autopsy case of typical cryptococcosis accompanied by leukemoid reaction. Tr. Soc. Path. Jap. 46, 413, 1957 (in Japanese)
39. ZELMAN, S., O'NEIL, R.H. & PLAUT, A. : Disseminated visceral torulosis without nervous system involvement. With clinical appearance of granulocytic leukemia. Am. J. Med 11, 658, 1951
40. ZIMMERMAN, L.E. & RAPPAPORT, H. : Occurrence of cryptococcosis in patients with malignant disease of reticuloendothelial system. Am. J. Clin. Path. 24, 1050, 1954
41. FITCHETT, M.S. & WEIDMAN, F.D. : Generalized torulosis associated with HODGKIN's disease. Arch. Path. 18, 25, 1934
42. ANDERSON, W.A. D. : Pathology 383, C.V. Mosby Co., St. Louis, 1958
43. KONDO, Y. *et al.* : Six autopsy cases of Cryptococcosis (mainly meningitis) during the past six years. Tr. Soc. Path. Jap. 47, 400, 1958 (in Japanese)
44. TSUBURA, Y., KITaura, K., IKEDA, Y., & TAMURA, K. : An autopsy case seems to be cryptococcal encephalitis. Tr. Soc. Path. Jap. 45, 354, 1956 (in Japanese)
45. BAKER, R.D. & HAUGEN, R.K. : Tissue changes and tissue diagnosis in cryptococcosis. A study of 26 cases. Am. J. Clin. Path. 25, 14, 1955
46. OKUDAIRA, M. : Histopathologic differential diagnosis of the mycosis. Jap. J. Clin. Path. Special issue No. 6, 169, 1957 (in Japanese)
47. KAWASHIMA, T., MIHASHI, S., & OTAKA, H. : Experimental and pathological studies on the establishment of mycotic changes. (Report III) Pathological changes and fungus findings in the experimental cryptococcosis. Tr. Soc. Path. Jap. 47, 398, 1958 (in Japanese)
48. SHIMOMURA, S. & KAWAI, K. : General infection due to *Cryptococcus neoformans*. Tr. Soc. Path. Jap. 45, 366, 1956 (in Japanese)
49. FREEMAN, W. : Torula infection of the central nervous system. J.F. Psychol. und Neurol. 43, 236, 1931
50. ZEMAN, W. und BEBIN, J. : Zur pathologischen Anatomie der Torula-Meningoencephalitis. Dtsch. Z. Nervenheilk. 168, 406, 1952
51. MOORE, M. : Morphologic variation in tissue of the organisms of the blastomycosis and the histoplasmosis. Am. J. Path. 31, 1049, 1955
52. SCHWARZ, J. : Giant forms of *Histoplasma capsulatum* in tissue explants. Am. J. Clin. Path. 23, 898, 1953
53. TUTTLE, J.G., LICHTWARDT, J.E. & ALTSHULER, C.H. : Systemic North American blastomycosis. Report of a case with small forms of *Blastomycetes*. Am. J. Clin. Path. 23, 890, 1953
54. WATTS, J.W. : Torula infection; a review and report of 2 cases. Am. J. Path. 8, 167, 1932
55. KLIGMAN, A.M. *et al.* : The HOTCHKISS-McMANUS stain for the histopathologic diagnosis of fungus diseases. Am. J. Clin. Path. 21, 86, 1951
56. KADE, H. *et al.* : Evaluation of staining techniques in the histologic diagnosis of fungi. Arch. Path. 59, 571, 1955
57. NASU, T. *et al.* : Autopsy cases of pan-mycosis. Sogo-Rinsho 4, 137, 1955 (in Japanese)
58. MIYAKE, M. & OKUDAIRA, M. : Pathology of the mycosis. Jap. J. Clin. Exp. Med. 31, 633, 1954 (in Japanese)
59. LILLIE, R.D. : Reactions of various parasitic organs in tissue to the BAUER, GRAM and GRAM-WEIGERT methods. J. Laborat. Clin. Med. 23, 76, 1947
60. GRIDLEY, M.F. : A stain for fungi in tissue sections. Am. J. Clin. Path. 23, 303, 1953
61. LILLIE, R.D. : The methenamine silver method. Histopathological technic and

- practical histochemistry. The Blakiston Company, Inc., New York, Toronto, 1947
62. LILLIE, R. D. : LILLIE's allochrome connective tissue method. Histopathological technic and practical histochemistry. The Blakiston Company, Inc., New York, Tront, 1947
63. MIKAMO, Y. : Clinic of mycosis. From the stand point of internal medicine. Reported in the 15th General Assembly of the Japan Medical Congress, April 1, 1959, Tokyo

# EXPLANATION OF FIGURES

## [Plate 1.]

- Fig. 1. Case 1. Lung. Showing granulomatous inflammation with marked infiltrations of lymphocytes, monocytes and macrophages. Several vacuolous spaces (→) with slightly stained organisms can be seen. Hematoxylin-eosin stain.  $\times 230$ .
- Fig. 2. Case 1. Lung. The alveolar spaces are filled with desquamated epithelium or macrophages. There can be seen many organisms. Gomori's methenamine silver stain.  $\times 230$ .
- Fig. 3. Case 1. The same as in Fig. 2. Fungi located extra-or intracellularly. A few of them show budding, but none of them possesses distinct capsule. Gomori's methenamine silver stain.  $\times 1480$ .
- Fig. 4. Case 1. Spleen. Several organisms forming a colony and possessing fairly wide spaces, but no inflammatory reaction can be seen. Hematoxylin-eosin stain.  $\times 330$ .
- Fig. 5. Case 1. Heart. Many organisms among the heart muscles. Gomri's methenamine silver stain.  $\times 230$ .
- Fig. 6. Case 1. The microphotograph shows a Malpighian corpuscle of kidney. In the markedly dilatated cavity of Bowman's capsule numerous spheric organisms can be seen. The glomurulus fell into severe atrophy. Gomori's methenamine silver stain.  $\times 230$ .
- Fig. 7. Case 2. Brain. In the leptomeningeal cavity there are marked infiltrations of lymphocytes, monocytes and macrophages. From these lesions the inflammatory cells are invading into cortex along the blood vessel. Hematoxylin-eosin stain.  $\times 100$ .
- Fig. 8. Case 2. Meninx. Some giant cells showing small vacuoles in its cytoplasma. Hematoxylin-eosin stain.  $\times 230$ .

## [Plate 2.]

- Fig. 9. Case 2. Brain. Granulomatous inflammation with a giant cell in the Virchow-Robin's space. Hematoxylin-eosin stain.  $\times 230$ .
- Fig. 10. Case 2. Brain. Cyst-like lesion in the perivascular space. Hematoxylin-eosin stain.  $\times 230$ .
- Fig. 11. Case 2. A necrotic lesion in the brain accompanied with infiltration of the microglias and a few lymphocytes. Hematoxylin-eosin stain.  $\times 230$ .
- Fig. 12. Case 3. The pleura showing marked thickening. Immediately under the pleura a grayish white localized lesion can be seen.
- Fig. 13. Case 3. Lung. Showing the granulomatous inflammation composed of lymphocytes, monocytes, macrophages, fibroblasts and a few giant cells. Hematoxylin-eosin stain.  $\times 230$ .
- Fig. 14. Case 3. Lung. Showing many organisms in the macrophages, no capsule can be detected. Gridley's stain.  $\times 1480$ .



- Fig. 15. Case 3. A colony of the large form fungi in the fibrously thickened pleura. The organisms stained faintly with hematoxylin. Hematoxylin-eosin stain.  $\times 150$ .

[Plate 3.]

- Fig. 16. Case 3. Pleura. Within the protoplasm of the fungi a nucleus-like droplets are seen, and stained with Sudan III deeply. Sudan III stain.  $\times 1480$ .  
 Fig. 17. Case 3. Pleura. Thick transparent capsule are clearly detected around the fungus body. India ink method.  $\times 1480$ .  
 Fig. 18. Case 4. Lung. A grayish white semitransparent localized lesion which gives a gelatinous aspect.  
 Fig. 19. Case 4. Lung. A giant cell ingesting large organisms in its cytoplasm is seen in the granulomatous tissue surrounding the necrotic lesion. Hematoxylin-eosin stain.  $\times 600$ .  
 Fig. 20. Case 4. Lung. Showing many spherical vacuolous spaces in the necrotic region and within these spaces many fungi can be seen. Hematoxylin-eosin stain.  $\times 150$ .  
 Fig. 21. Case 4. Lung. Large spherical fungi possessing a thick capsule stained deep red Gridley's stain.  $\times 230$ .  
 Fig. 22. Case 4. Lung. In the same necrotic lesion as in the Fig. 21 these organisms do not possess the capsule. Gridley's stain.  $\times 600$ .

[Plate 4.]

- Fig. 23. Case 4. Lung. Microgram shows a germ tube projecting from a large spherical organism. Gridley's stain.  $\times 1480$ .  
 Fig. 24. Case 5. Lung. Several localized lesions as big as millet with grayish white color whose center is yellow can be observed on the subpleural region in the lung.  
 Fig. 25. Case 5. Lung. Microgram showing the marked proliferation of fibroblasts and the infiltration of lymphocytes and monocytes. Two organisms can be seen among the proliferated fibrocytes ( $\rightarrow$ ). Hematoxylin-eosin stain.  $\times 230$ .  
 Fig. 26. Case 5. Lung. In the center of the nodular lesion many lymphocytes and polymorpho-nuclear leucocytes have agglomerated. In the giant cells or among the cells several organisms can be seen. Hematoxylin-eosin stain.  $\times 230$ .  
 Fig. 27. Case 5. Lung. Several *C. neoformans* ingested in the macrophages and gelatinous capsule can not be seen. Lillie's alochrome stain.  $\times 1480$ .  
 Fig. 28. Case 5. *C. neoformans* isolated from the lung and cultured 6 days on the Sabouraud's medium. Organisms possess a thick cell-wall and the gelatinous capsule can not be seen. India ink method.  $\times 1480$ .  
 Fig. 29. (A, B). Case 6. Several organisms on the blood smear. Giemsa stain.  $\times 1480$ .  
 Fig. 30. Case 6. Axillary lymph node. The reticulum cells are proliferated and swollen markedly, some of them form giant cells, and many of them ingest numerous organisms. Hematoxylin-eosin stain.  $\times 330$ .

[Plate 5.]

- Fig. 31. Case 4. Lung. Thick capsule relatively deeply stained with hematoxylin. Hematoxylin-eosin stain.  $\times 1480$ .  
 Fig. 32. Case 4. Lung. Radial aspect of the capsule seems to be only an artifact. Hematoxylin-eosin stain, fixed with formalin.  $\times 1480$ .

- Fig. 33. Case 6. Axillary lymph node. Showing homogeneous pattern of the capsules stained with mucicarmine. Mucicarmine stain, fixed with Carnoy's solution.  $\times 1480$ .
- Fig. 34. Case 2. Meninx. Sometimes, organisms possess a thick capsule even ingested within the giant cell. Lillie's alochrome stain.  $\times 1480$ .
- Fig. 35. Case 4. Lung. With Gram stain, in (a) and (b) only the cell wall is positive, but capsules and cytoplasmas are negative. (c) is intensely stained with fuchsin on the whole. Gram stain, Goodpasture's method.  $\times 1480$ .

Plate 1

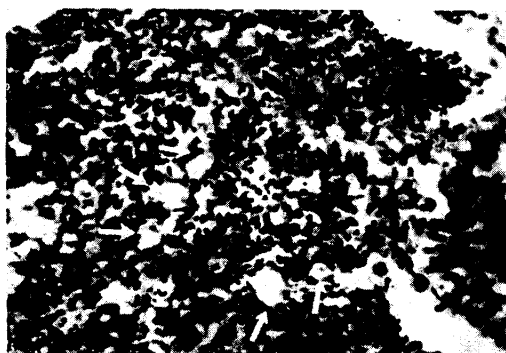


Fig. 1.

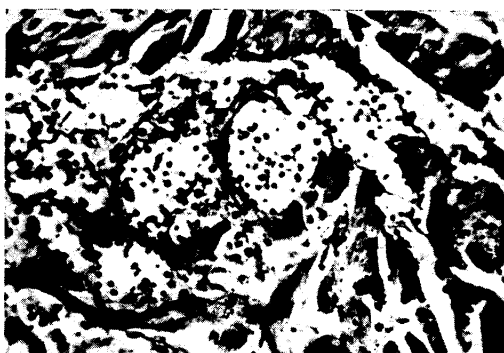


Fig. 5.

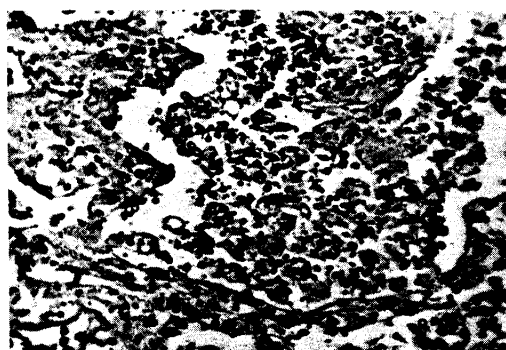


Fig. 2.

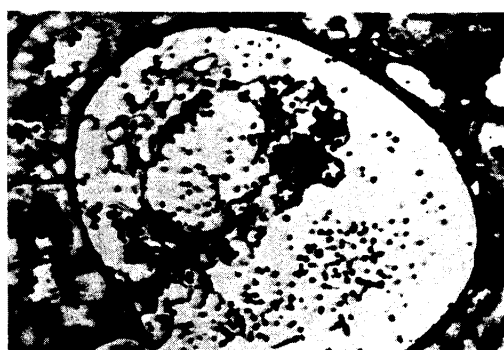


Fig. 6.

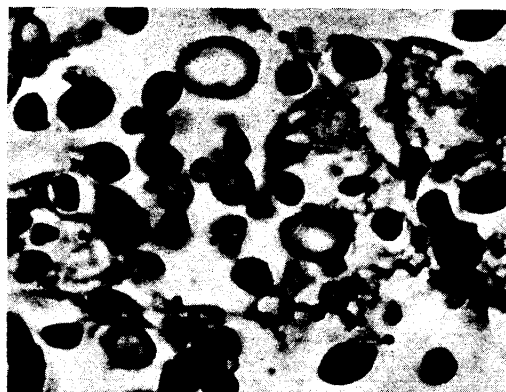


Fig. 3.



Fig. 7.

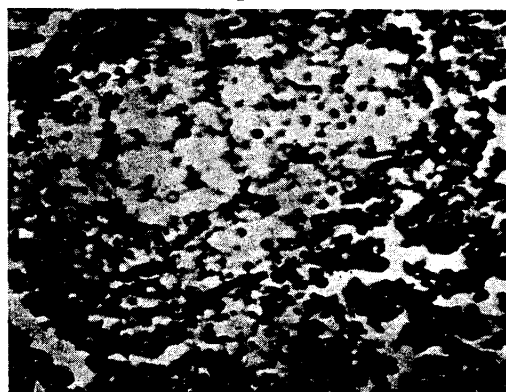


Fig. 4.



Fig. 8.

**Plate 2**



Fig. 9.

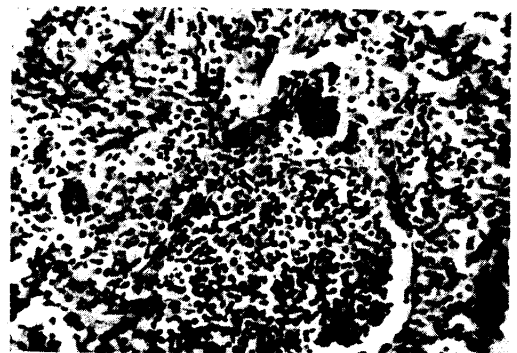


Fig. 13.

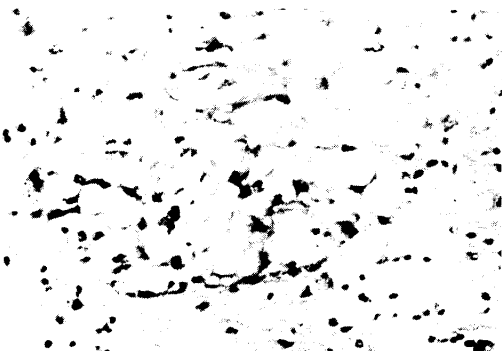


Fig. 10.



Fig. 14.



Fig. 11.



Fig. 12.



Fig. 15.

Plate 3



Fig. 16.

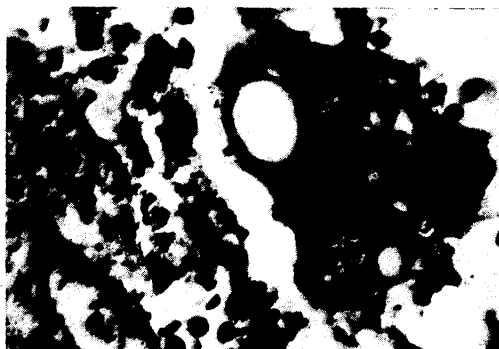


Fig. 19.

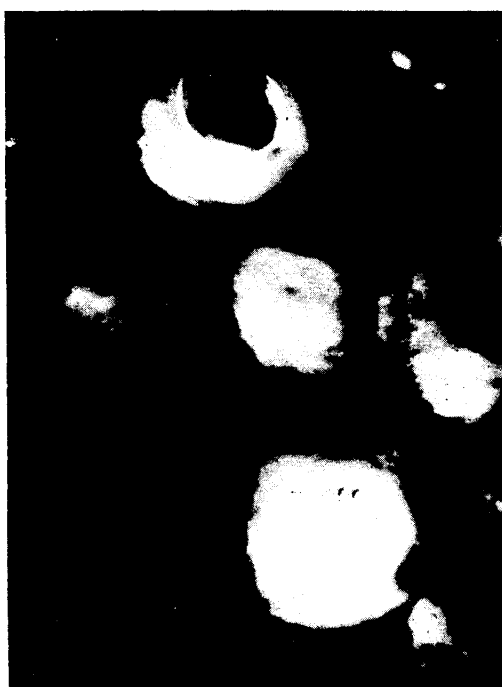


Fig. 17.

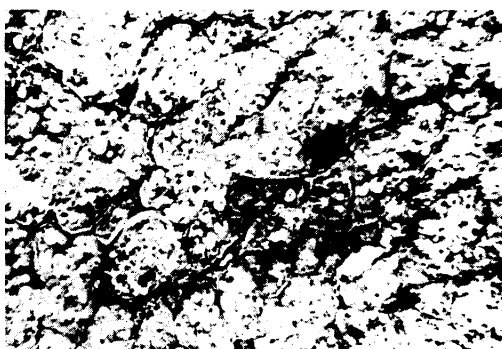


Fig. 20.

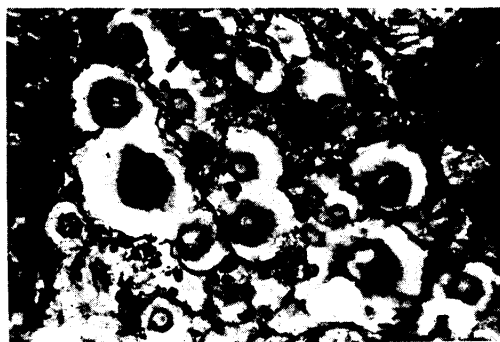


Fig. 21.

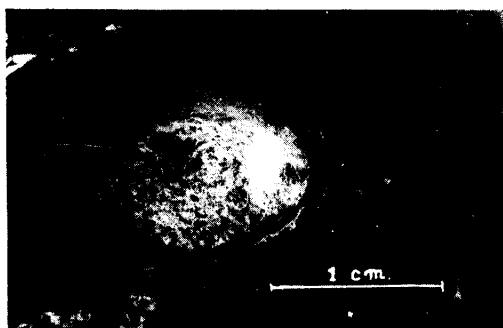


Fig. 18.



Fig. 22.

**Plate 4**

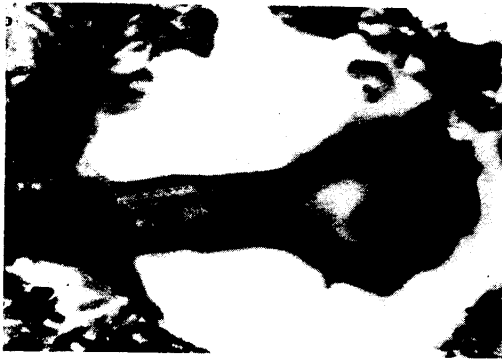


Fig. 23.



Fig. 27.



Fig. 24.

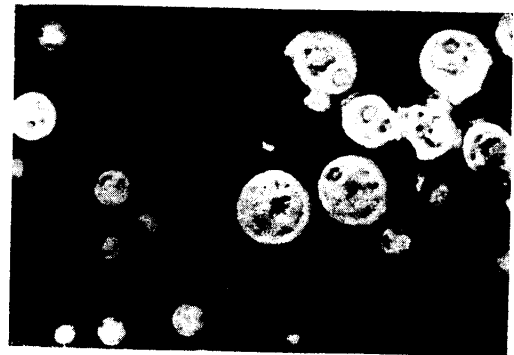


Fig. 28.

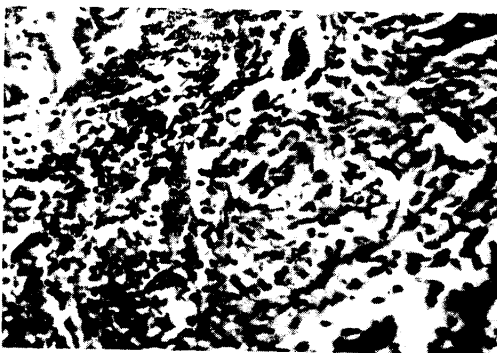


Fig. 25.



Fig. 29. a

b

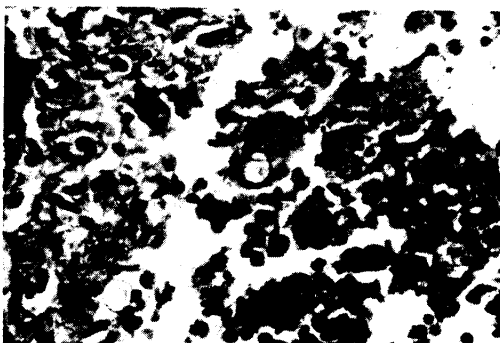


Fig. 26.



Fig. 30.

Plate 5



Fig. 31.



Fig. 34.

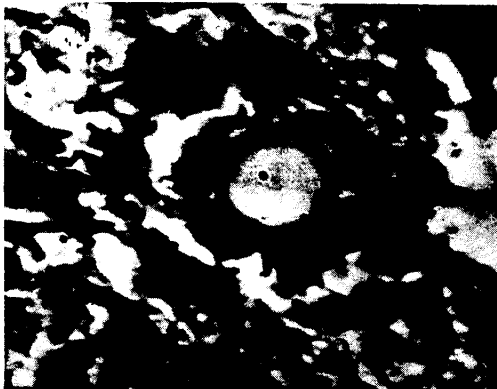


Fig. 32.



Fig. 33.

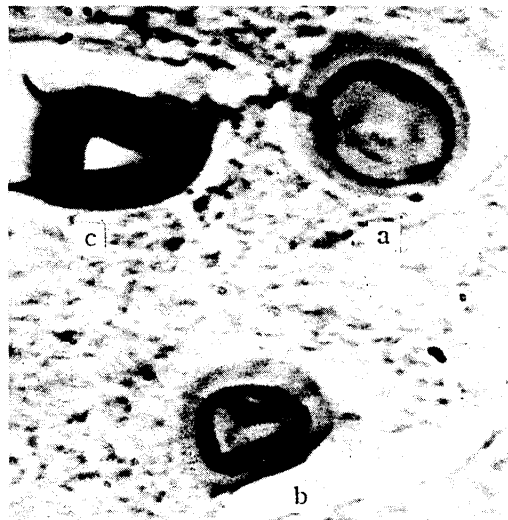


Fig. 35.